In-Situ Water Quality Monitoring for Resource-Constrained Areas

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ABSTRACT

Water-borne pathogens reduce the quality of people's lives globally, and exposure to these unsafe water supplies is the cause of death for 1.7 million people every year [1]. Areas of the world where it is difficult to manage water safety are especially affected. Though there are standardized water quality measurements worldwide, many of these standardized methods are infeasible in the regions that are most affected as they can take up to 24 hours and require specialized, potentially unsustainable equipment and trained operators [2]. Fluorometers eliminate many of these barriers by providing real-time measurement of both presence and number of thermotolerant (fecal) coliforms (TTCs) in water samples. Fluorometers could be an improvement on the current WHO standards, but current fluorometers are too expensive for in-field use, often costing upwards of $20,000 [3]. This thesis presents a low-cost fluorometer that passively and accurately collects real-time water quality measurements. Our device measures a fluorescent signal called tryptophan-like fluorescence (TLF) produced by bacteria when exposed to light at a specific frequency. Our device exploits this phenomenon to compute the quantity of bacteria in the sample from the measured fluorescence. We hypothesize that this device will meet the current WHO standards for water quality measurement accuracy and will be cheaper, easier to use, and more sustainable than the current methods.

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INTRODUCTION

Water-borne pathogens and unsafe water supplies are the cause of death for 1.7 million people every year [1]. Many of these people live in the developing world where it can be difficult to manage water safety. The World Health Organization mandates that many countries use standardized water quality measurements, but these measurements can take up to 24 hours and require specialized, potentially unsustainable equipment and trained operators [2]. For water-borne pathogens to be eliminated and safe water available to all, a more efficient water quality measuring standard must be developed.

While water quality detection methods currently available are diverse, fluorometry is emerging as one of the most effective methods [4]. Fluorometers measure fluorescence following excitation, focusing on wavelengths that indicate presence of specific organic compounds. Fluorometers can provide real-time prediction of both presence and number of thermotolerant (fecal) coliforms (TTCs) and other fecal indicator bacteria (FIB), including *E. coli*, in water samples, because this group of microbes has been shown to correlate with specific classes of fluorescent compounds. FIB are an important indicator of human pathogens [5]. This makes a fluorimeter an ideal water quality measuring device by reducing the large latency of water testing by current WHO guidelines [3]. Sorensen et al. found that using tryptophan-like fluorescence (TLF), a signal specific to organic molecules associated with FIB, to detect TTC's was highly accurate and could be used to correlate the elimination of open defecation and the pollution of ground water by sewage [5]. It was also found that TLF was the most accurate of several methods used to detect TTC's [3]. However, the fluorometers used in these studies are too expensive and impractical for field use, which would be required in rapid assessments. Wigton et al. demonstrated that creating a low-cost fluorometry device is possible by designing a device

that can be built for less than 100 dollars for middle and high school chemistry labs [8]. The low-cost device created in that study only had educational applications, and is not able to detect TTC's. This thesis combines the accessibility of low-cost fluorometry with the robust performance of more expensive laboratory equipment.

We present a low-cost fluorometer that passively and accurately collects real-time water quality measurements. Our device measures a fluorescent signal called tryptophan-like fluorescence (TLF). When bacteria are exposed to light at a wavelength of 280nm, they fluoresce at 350nm. This device first excites a water sample with 280 nm light, then records the amount of 350nm light emitted to compute the quantity of bacteria in the sample. We hypothesize that this device meet the current WHO standards for water quality measurement accuracy and will be cheaper, easier to use, and more sustainable than current methods.

LITERATURE REVIEW

Thermotolerant Coliforms

One of the most important aspects of the effort to provide clean water to all people is accurately detecting the quality of water. The presence of thermotolerant coliforms (TTC) are one of the main indicators of how fit water is for human consumption. Thermotolerant coliforms are defined as coliforms that thrive in environments around 44.5 degrees Celsius. According to the World Health Organization, they are important faecal indicators as they can survive in the bile salts of the human gut and are often present in human feces [7]. The device proposed in this research will detect the presence of these thermotolerant coliforms in water in real-time.

Methods of Detection

Over time, there have been many different approaches to the detection of thermotolerant coliforms. In the 1940s, the indole test was the most popular presence/absence test for the detection of these coliforms as they are indole positive [8]. However, these indole tests were not specific enough because they detected total coliforms instead of only the thermotolerant coliforms that are disease causing. In the 1950s, membrane filtration replaced indole tests as the most popular microbial detection test. Though this new form of detecting coliforms was more practical than the indole tests, it often missed *E. coli* and thermotolerant coliforms because it focused on the ability of the bacteria to produce gas from the formation of lactose, which these dangerous bacteria do not always do [9]. More recently Colilert techniques are used to detect these coliforms, as they use a less harsh substrate that allows more of the coliforms to be successfully detected [10]. Another modern method of detection includes using polymerase chain reaction (PCR), which can amplify pieces of target DNA in a sample for non-culturable samples [11].

Tryptophan-Like Fluorescence

Proteins that are folded have a certain fluorescence depending on their individual aromatic residues. Protein fluorescence is mainly based on the presence of tryptophan residues. When tryptophan residues are hit with light at a wavelength of 280nm, they emit light at approximately 350nm [12]. This unique property can be used to identify the conformational state of a protein. It is particularly useful for determining the presence of biological materials in water and can be used as an indicator for the presence of thermotolerant coliforms. The device proposed in this research will exploit this unique property of thermotolerant coliforms to detect their existence in a water sample.

Current Fluorometry Methods

Fluorometers are currently used to detect thermotolerant coliforms, but the models currently available are extremely expensive and designed to be used by a trained professional in a laboratory setting. Sorensen et al tested a fluorometer measuring tryptophan like fluorescence (TLF) versus some of the other previously discussed methods of detecting thermotolerant coliforms to determine which method of detection was more accurate [3]. They could determine that the fluorometer provided the best detection of the coliforms. In another study by Sorensen et al, a fluorometer was used to measure tryptophan like fluorescence during an attempt to determine the relationship between ground water pollution and the existence of open defecation [3]. They found that the TLF device could accurately correlate the existence of open defecation with the amount of TTC in the water.

Low Cost Fluorometry

While the fluorometers used in the above-mentioned studies were too expensive and bulky for field use, creating a low-cost version of a fluorometer has been done before. Wigton et al

designed a fluorometer that was cheap and easy enough to build that it could be made for educational purposes in high school and college classrooms [6]. Their design focused on fluorometry of certain wavelengths to reduce the complexity of the device. Though this device is very suitable for educational settings, it does not provide the same water quality applications of the cost fluorometers used in the water studies.

Proposed Device

The device proposed in this paper will combine the accessibility of the educational fluorometers with the water quality uses of the expensive fluorometers into a device that can be used for real-time water quality monitoring in low-resource areas. It will consist of an LED that emits light at 280nm, a sensor that records light at 350nm, and a microcontroller to transmit the data remotely. The device will use the amount of 350nm light fluorescing from the thermos tolerant coliforms in order to detect the presence and the amount of bacteria in the water. The device will have a flow-through chamber for water to move through passively and will be deployable in the field and monitored from afar. This TLF device will omit the need for the 24 hr+, expensive, and bulky water quality tests that currently exist. Because of this new water quality measuring device, the global goal of clean water for all will be reached in a much faster and more efficient way.

METHODS

Device Creation

The device was created using two circuits. All the components of the circuits were selected to balance signal amplification and cost. One circuit powers an LED and the other powers a sensor that detects fluorescence from the bacteria in the water. Selecting the correct photodiode sensor was crucial to allow this device to handle the low signal-to-noise ratios of the measurements.

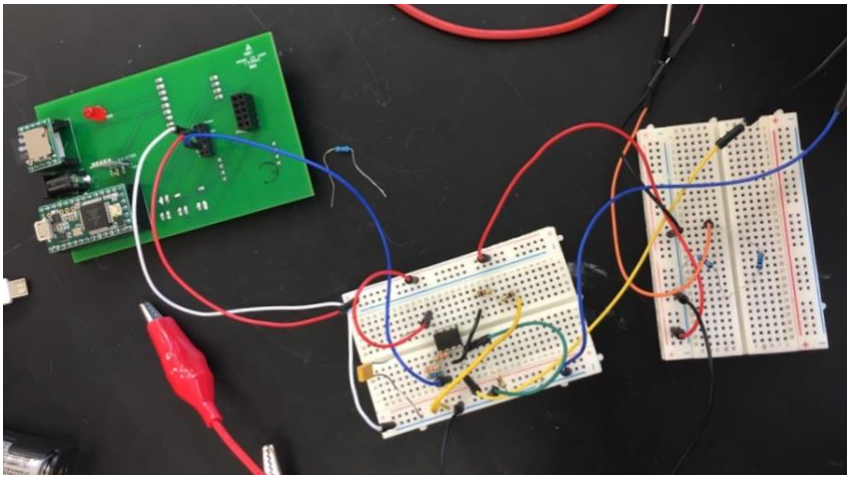

Figure 1. Sensor and LED circuit with Arduino microcontroller.

Proof of Concept Testing

To conduct proof of concept testing, a water sample with a high quantity of TTC's was collected from the primary affluent section of a wastewater treatment plant in Atlanta. The TLF observed by the device from that water was then compared to that of laboratory distilled water. The goal of this experiment was to determine if the fluorometer is able work as a presence/absence detector for TCC's, not to determine if it could quantify the exact amount of TTC's in a source. Each sample of water was tested in a darkened container to ensure that ambient light does not interfere with the fluorometry measurements. The fluorescence was measured from each sample ten times and averaged.

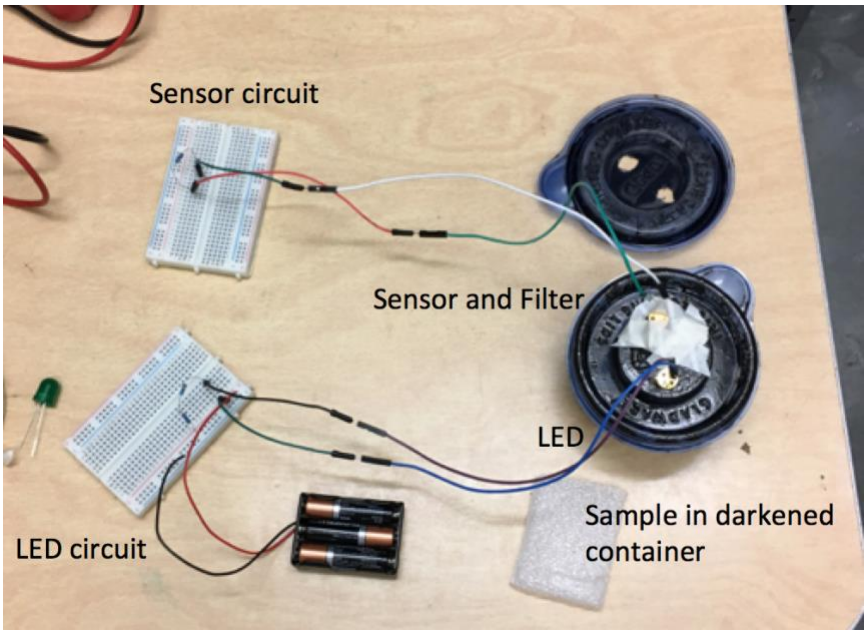

Figure 2: Experimental set-up for the Proof of Concept Testing

A two-sample t-test was conducted in R to test for a significant difference between the voltage values collected from the primary affluent sample versus those of the DI water sample.

Device Calibration

After proof of concept testing was completed, the device was tested for its ability to detect different quantities of TTC's, rather than just whether any TTC's were present. *E. coli* colonies were grown and diluted to make a series of plates with *E. coli* concentrations ranging from 10^{-1} to 10^{-9} (Figure 3).

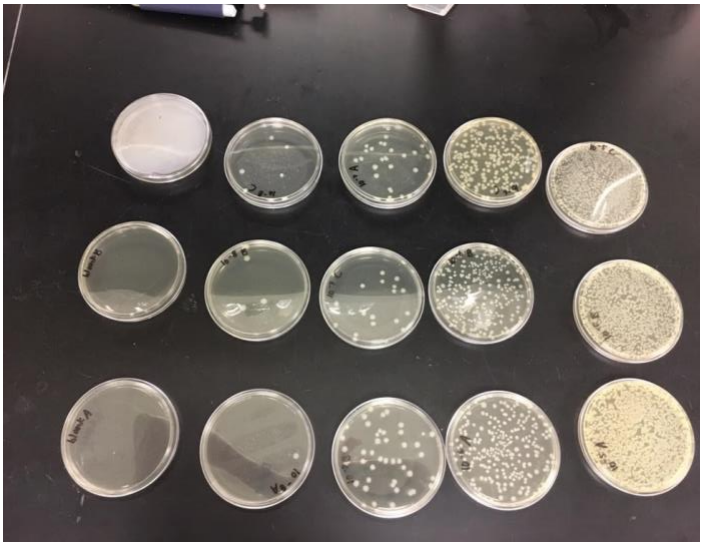

Figure 3. *E. coli* plates at different dilutions.

To test the fluorescence of different *E. coli* concentrations, a sample from the *E. coli* plates at different concentrations was swabbed. This swab was then mixed with DI water and placed in a quartz cuvette. The cuvette was placed in an advanced device set up where the detector and LED were held in place next to the quartz cuvette with Velcro placements (Figure 4). This entire set-up was placed inside a light proof box. The output from the sensor was recorded for each *E. coli* concentration level. The sensor output was read both with the Arduino microcontroller and with a digital multimeter.

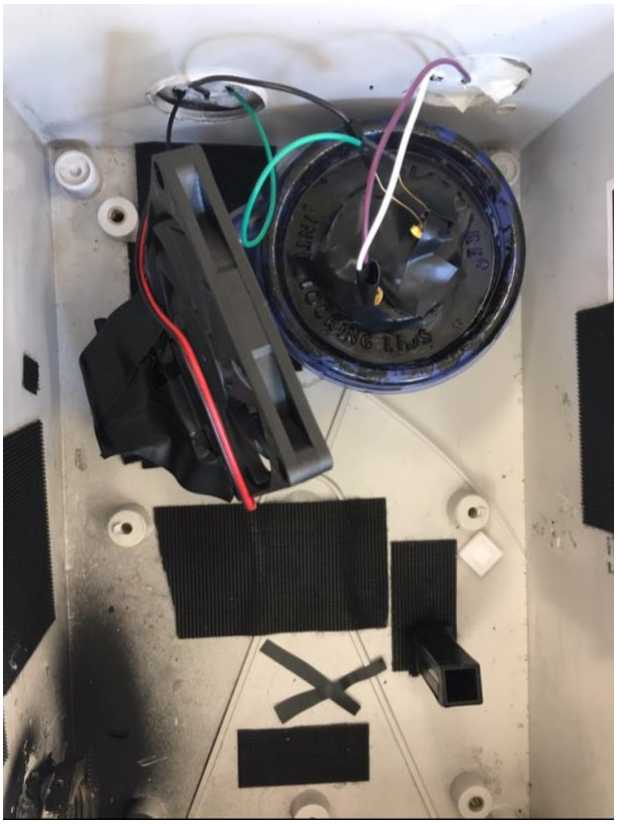

Figure 4. Experimental set-up for calibration testing

RESULTS

Proof of Concept Testing

To determine if the fluorometer can accurately detect a difference between samples contaminated by TTC's and samples not contaminated, a paired t-test was conducted between the voltage collected from each sample.

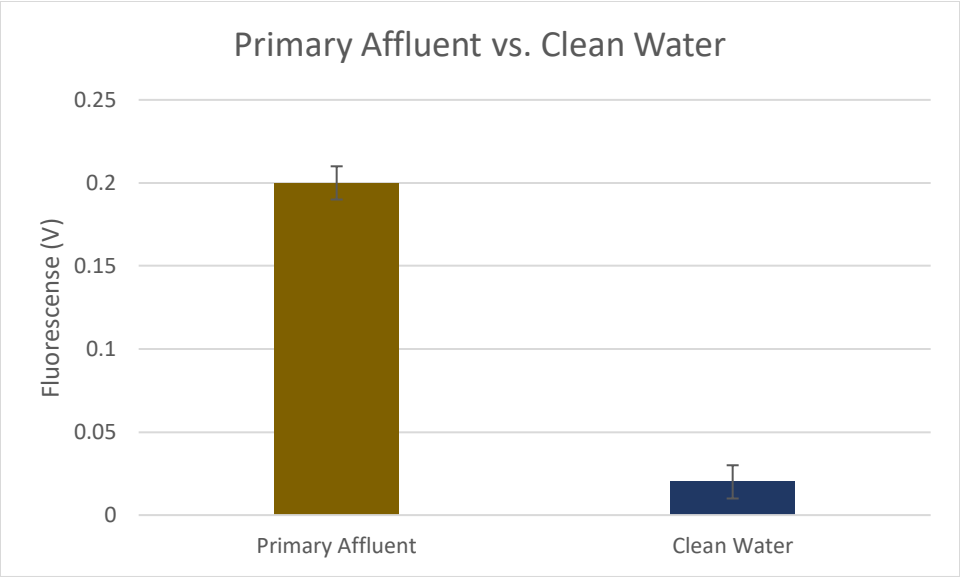

Figure 5. Experimental results from the proof of concept testing with the primary affluent versus clean water. The voltage was not significantly greater, though it was close to the level of significance, for the primary affluent versus the clean water. This was demonstrated with a two-sample t-test conducted in R with alpha= 0.05 (t=2.5755, df=2.2523, p-value=0.05481).

Device Calibration

To determine if the fluorometer could accurately detect the difference between different concentrations of *E. coli*, an ANOVA test was conducted in R between the voltage output between each *E.coli* concentration level.

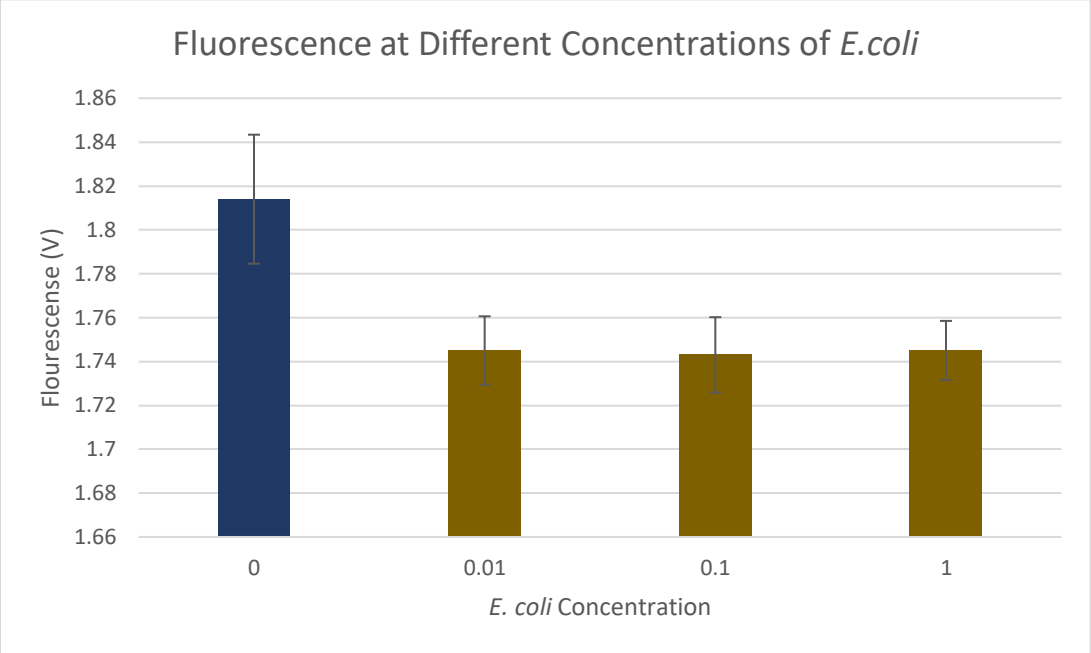

Figure 6. Experimental results from the device calibration testing with *E. coli* at different concentrations and distilled water. The voltage was not significantly different for each *E. coli* concentration, demonstrated by an ANOVA test conducted in R with alpha= 0.05 (df of treatment=1, df of residuals=2, F=0.32, p-value= 0.628)

DISCUSSION

Tryptophan-like fluorescence can be used as a low cost method of detecting a difference between water contaminated by TTC's and water not exposed, but it cannot currently detect the differences between different levels of contamination by TTC's.

Proof of Concept Testing

Proof of concept testing demonstrated that this fluorometry device can detect the difference between a water sample contaminated by TTC's and a clean sample of DI water. However, there were confounding variables for this section in the experiment that were hard to control and it is important for future water quality research to develop a comprehensive method of controlling for these variables. One challenge with using the primary affluent sample from the wastewater treatment plant was that many other things besides TTC's were most likely present in the water. The combination of contaminants in the water increased the turbidity of the sample. Because the researchers could not be certain of all the other contaminants present in the water sample or of the effect of high turbidity of the sample on TLF, it is possible that these variables interfered with proof of concept testing. It is important for future water quality device testing that the scientific community develops a better understanding of the way turbidity influences water samples.

Device Calibration

The device currently cannot detect the differences between different concentrations of *E. coli* in a water sample. This could be due to many different components of the device, and especially due to the sensitivity of the photodiode sensor. Because the level of TTC's this device must detect is so low, minute deviations in device setup during testing may have disrupted the accuracy of the voltage readings. A more robust apparatus construction is required for future

calibrations. Currently, work is being conducted that uses different photodetectors to detect the varying concentrations of *E.coli* in a sample.

CONCLUSION

Low-cost fluorometry is a promising new method for water quality detection. The work in this thesis shows that the device in its current iteration can accurately detect presence-absence of thermotolerant coliforms in water. Though it cannot currently detect differences between *E.coli* concentrations on a logarithmic scale, ongoing and future work is implementing new signal amplification methods with the goal of detecting the smaller differences between fluorescence at the scale of different *E.coli* concentrations. New sensor technology that allows for greater photo-sensitivity at a low-cost scale is being implemented in new iterations of the device. In the future, this low-cost water quality detection technology could potentially be employed for specific thermotolerant coliform detection.

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